



A critical review on anaerobic co-digestion achievements between 2010 and 2013



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ABSTRACT

Anaerobic digestion is a commercial reality for several kinds of waste. Nonetheless, anaerobic digestion of single substrates presents some drawbacks linked to substrate characteristics. Anaerobic co-digestion, the simultaneous digestion of two or more substrates, is a feasible option to overcome the drawbacks of mono-digestion and to improve plant's economic feasibility. At present, since 50% of the publication has been published in the last two years, anaerobic co-digestion can be considered the most relevant topic within anaerobic digestion research. The aim of this paper is to present a review of the achievements and perspectives of anaerobic co-digestion within the period 2010–2013, which represents a continuation of the previous review made by the authors [3]. In the present review, the publications have been classified as for the main substrate, i.e., animal manures, sewage sludge and biowaste. Animal manures stand as the most reported substrate, agro-industrial waste and the organic fraction of the municipal solid waste being the most reported co-substrate. Special emphasis has been made to the effect of the co-digestion over digestate quality, since land application seems to be the best option for digestate recycling. Traditionally, anaerobic co-digestion between sewage sludge and the organic fraction of the municipal solid waste has been the most reported co-digestion mixture. However, between 2010 and 2013 the publications dealing with fats, oils and greases and algae as sludge co-substrate have increased. This is because both co-substrates can be obtained at the same wastewater treatment plant. In contrast, biowaste as a main substrate has not been as studied as manures or sewage sludge. Finally, three interdisciplinary sections have been written for addressing novelty aspects in anaerobic co-digestion, i.e., pre-treatments, microbial dynamics and modeling. However, much effort needs to be done in these later aspects to better understand and predict anaerobic co-digestion.

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Abbreviations: AcoD, anaerobic co-digestion; AD, anaerobic digestion; BMP, biomethane potential test; CM, cow manure; CSTR, continuous stirred tank reactor; C/N, carbon-to-nitrogen ratio; DGGE, denaturing gradient gel electrophoresis; EU, European Union; FISH, fluorescence in situ hybridization; FOG, fat, oil and grease; FVW, fruit and vegetable waste; FW, food waste; GLY, glycerol; HRT, hydraulic retention time; IBR, induced bed reactor; LCFA, long chain fatty acids; MSW, municipal solid waste; OLR, organic loading rate; OLR_{FOG}, FOG organic loading rate; OFMSW, organic fraction of municipal solid waste; OMW, olive mill waste; PCR, polymerase chain reaction; PS, primary sludge; PM, pig manure; SHW, slaughterhouse wastes; SMP, specific methane production; SS, sewage sludge; TOC, total organic carbon; TS, total solids; TSS, total suspended solids; T-RFLP, terminal restriction fragment length polymorphism; WAS, waste activated sludge; US, ultrasound pre-treatment; VFA, volatile fatty acid; VS, volatile solids; VSS, volatile suspended solids; WW, wastewater; WWTP, wastewater treatment plant

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1. Introduction

Anaerobic digestion (AD) is a biological treatment performed in the absence of oxygen to stabilize organic matter while producing biogas, a mixture formed mainly of methane and carbon dioxide. The oldest and more widespread application of AD is the treatment of sewage sludge (SS). AD experienced an important growth after the first energy crisis in the 1970s, especially with the appearance of immobilized biomass systems to treat soluble effluents, and now it can be considered a mature technology [1,2]. Nonetheless, AD of single substrates (mono-digestion) presents some drawbacks linked to substrate properties. For instance, (i) SS is characterized by low organic loads, (ii) animal manures have low organic loads and high N concentrations, that may inhibit methanogens, (iii) the organic fraction of municipal solid waste (OFMSW) has improper materials as well as a relatively high concentration of heavy metals, (iv) crops and agro-industrial wastes are seasonal substrates, which might lack N, and (v) slaughterhouse wastes (SHW) include risks associated with the high concentration of N and/or long chain fatty acids (LCFA), both potential inhibitors of the methanogenic activity. Most of these problems can be solved by the addition of a co-substrate in what has been recently called anaerobic co-digestion (AcoD).

AcoD, the simultaneous AD of two or more substrates, is a feasible option to overcome the drawbacks of mono-digestion and to improve the economic viability of AD plants due to higher methane production [1]. Initially, because of the research perspective, AcoD focused on mixing substrates which favor positive interactions, i.e. macro- and micronutrient equilibrium, moisture balance and/or dilute inhibitory or toxic compounds [3]. Under these circumstances, $1 + 1 > 2$ may be achieved, that means, co-digestion is producing more methane than the addition of the methane produced in both single digestions. However, nowadays, because of the industrial outlook and since the improvement of methane production is mainly a consequence of the increase in the organic loading rate (OLR) rather than synergisms, all kinds of mixtures are considered and used. Actually, the transport cost of the co-substrate from the generation point to the AD plant is the first selection criteria. Despite this fact, it is still important to choose the best co-substrate and blend ratio with the aim of favoring synergisms, dilute harmful compounds, optimize methane production and not disrupt digestate quality.

As illustrated in Fig. 1, AcoD publications have experienced a dramatic increase in the last years. In fact, at the present time, AcoD can be considered the most relevant topic within anaerobic digestion research. As can be seen, 50% of the overall papers have been published between 2012 and 2013, whereas 75% of them have been published in the period 2009–2013.

Examining the papers published between 2010 and 2013, it appears that the most frequent main substrates are animal manures (54%), SS (22%) and the OFMSW (11%). At the same time, the most used co-substrates are industrial waste (41%), agricultural waste (23%) and municipal waste (20%) (Fig. 2).

The aim of this paper is to present a review of the achievements and perspectives of anaerobic co-digestion within the period 2010–2013 (up to the 13th World Congress on Anaerobic Digestion), which represents a continuation of the previous review made by the authors [3]. The use of manures, sewage sludge, and organic fraction of municipal solid waste as main substrates, either full-scale or research experiences, is discussed throughout the paper. Furthermore, the review also pays attention to other aspects like pretreatments, digestate quality, microbial community dynamics, and modeling.

2. Animal manures as a main substrate

In the rural sector, AD has been established as an important economical alternative, specifically as a renewable energy source; hence animal manures have become an important raw material [3]. Nonetheless, manures are often associated with poor methane yields. AcoD of manures with other substrates has been applied as a cost-effective alternative to improve process efficiency and consequently make plants economically feasible [4–6]. Two main models can be chosen for the implementation of agriculture-based

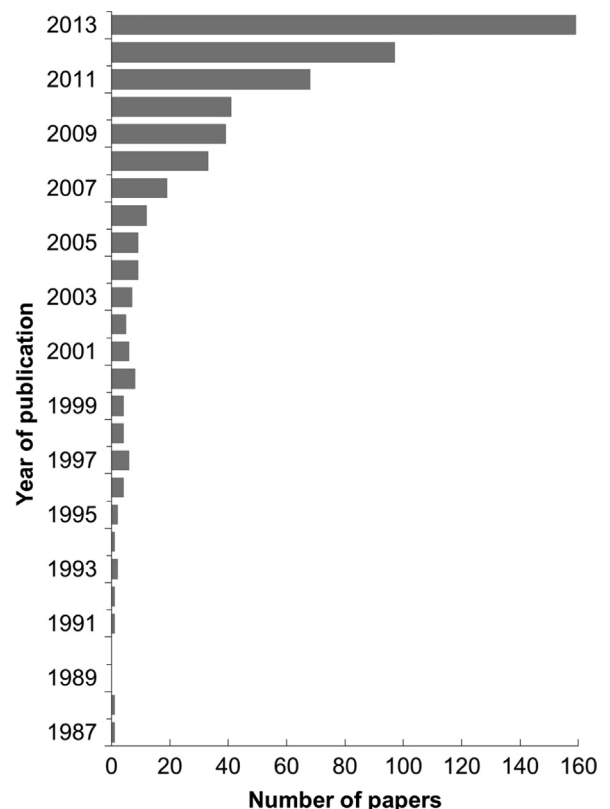


Fig. 1. Evolution of number of papers published with the words co-digestion or co-digestion in its title.

AD plants [7,8]: (i) centralized plants, which co-digest manures collected from several farms together with organic residues from industry and township, and (ii) on-farm plants, which co-digest manure with other farm waste and, increasingly, energy crops. Germany is the undisputed leader in the application of on-farm AD systems with over 4000 plants currently in operation [8]. Nevertheless, co-digestion systems have also been widely implemented in other countries. For example, Sweden with more than 200 plants (10 of them are centralized, while the remaining are farm-scale plants), Denmark with 20 centralized plants and more than 60 farm-scale plants, or Italy with about 500 AD plants [9–11]. Beyond methane production improvement, other options to enhance agriculture-based AD plants profitability include the following [10]: (i) gaining further incomes from the digestate sale as fertilizer, (ii) locating the power plants near to heat demand facilities, (iii) integrating the power plants into farms, in order to reduce operational costs, or (iv) integrating biomass transport into the business of either biomass producers or power plant operators.

2.1. Traditional and new co-substrates for animal manure co-digestion

In AcoD publication animal manures refer mainly to pig manure (PM) and cow manure (CM), since less attention has been paid to other livestock (goat, horse, etc.) and poultry manure. Regarding the co-substrates used, agro-industrial waste stands as the most applied co-substrate (47%), followed by OFMSW (12%),

crude glycerol (GLY) (9%), cheese whey (5%), and olive mill waste (OMW) (4%) (Fig. 3).

As can be observed, suitable co-substrates for manures are C-rich substrates and, when possible, with large amounts of easily biodegradable organic matter. These co-substrates are characterized by high C/N ratio, poor buffer capacity, and, depending on their biodegradability, the capacity of producing large amounts of volatile fatty acids (VFA) during the AD process. In contrast, manures have high buffer capacities and low C/N ratios, where ammonia concentrations usually surpass the requirements for microbial growth and may become inhibitory for methanogens [3,12,13]. Therefore, AcoD between manures and C-rich wastes overcome these problems by maintaining a stable pH, within the methanogens range, due to their inherent high buffering capacity and reducing the ammonia concentration by dilution while enhancing methane production [12,13]. Typically, the decisions on the ratio between wastes had been simplified to the optimization of the C/N ratio, but, as mentioned in Section 1, the right combination of several other parameters in the mixture is also relevant, e.g., macro and micronutrients, pH and alkalinity, inhibitors and toxic compounds, biodegradable organic and dry matter [14]. Some studies have been carried out with the aim of investigating the best performance of AcoD based on C/N ratio optimization [15,16]. For instance, Wu et al. [17] reported the best AcoD performance when a mixture between PM and cereal straws had a C/N of 20; Panichnumsin et al. [18], who co-digested cassava pulp and PM, reported the maximum methane yield when the feedstock contained a C/N ratio of 33; while, Zhang et al. [19] found an optimum C/N ratio of 16 when treating CM and OFMSW.

Although agro-industrial wastes are the most convenient co-substrates for manures, the need to overcome its seasonality and further improve the digesters methane production have raised interest over biodegradable industrial wastes and other substrates rich in biodegradable organic matter (Table 1).

Among them, GLY, by-product of the biodiesel production, has stood out as an ideal co-substrate because of its high theoretical methane production ($0.43 \text{ m}^3 \text{ CH}_4 \text{ kg}^{-1}$), biodegradability ($\sim 100\%$) and purity [13,25,26]. Other industrial by-products investigated with promising results are cheese whey [21,27], sugar by-products [28,29], OMW [22,30] and SHW [31,32]. However, when dealing with industrial wastes the biggest concern is the little knowledge about the possible presence of compounds that can become inhibitory to anaerobic biomass, especially methanogens, if a certain dose is exceeded [33]. Even more, the quality and composition of industrial waste depend on the raw matter origin, the chemical process used to obtain the main product and, if

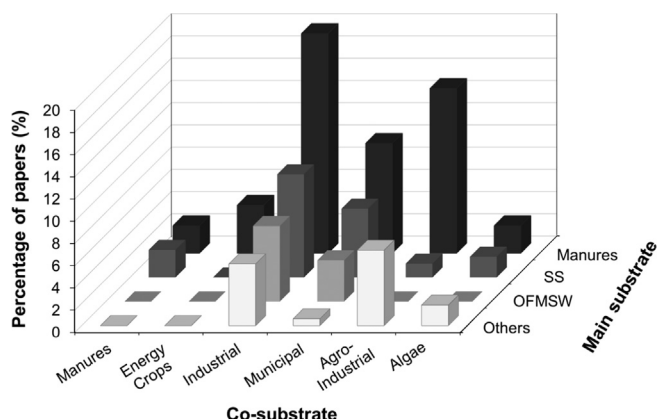


Fig. 2. Main substrates and co-substrates in co-digestion papers in the period 2010–2013.

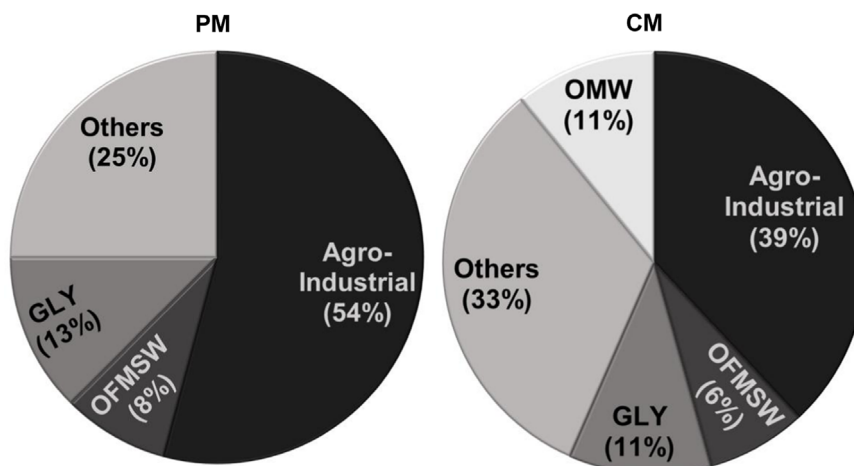


Fig. 3. Co-substrates distribution in research studies depending on the main substrate: PM and CM.

Table 1
Co-digestion of new co-substrates using CM and PM as main substrate.

Substrates	Mixture ratio	Digester configuration	T [°C]	OLR [$\text{kg}^{\text{a}} \text{m}^{-3} \text{d}^{-1}$]	SMP [$\text{m}^3 \text{CH}_4 \text{kg}^{-1\text{b}}$]	Improvement ^c	Reference
CM:Distillery WW	81:19 (wet basis)	Batch	37	–	0.12 ^d (VS)	–	[27]
CM:OMW	3:1 (wet basis)	CSTR	37	5.50 (COD)	0.18 (VS)	$\times 1.74$	[22]
			55	5.50 (COD)	0.21 (VS)		
CM:Sugar beet by-products	50:50 (wet basis)	CSTR	55	5.00 (VS)	0.24 (VS)	$\times 1.12$	[28]
CM:GLY	95:5 (wet basis)	CSTR	35–37	1.90 (VS)	0.82 ^d (VS)	$\times 3.05$	[35]
	90:10 (wet basis)			2.30 (VS)	0.83 ^d (VS)	$\times 3.09$	
CM:Cheese whey	35:65 (wet basis)	CSTR	35	2.42 (VS)	0.38 (VS)	$\times 3.49$	[45]
PM:GLY	96:4 (wet basis)	CSTR	35	1.90 (VS)	0.78 ^d (VS)	$\times 1.56$	[13]
CM:OMW	80:20 (wet basis)	CSTR – 2 stage	35	3.63 (COD)	0.25 (COD)	–	[30]
CM:Cheese whey	50:50 (wet basis)	CSTR – 2 stage	35	1.7 (COD)	0.26 (VS)	–	[46]
CM:GLY	94:6 (TS basis)	CSTR IBR	55	5.41 (COD)	0.60 (VS)	–	[26]
			55	7.25 (COD)	0.59 (VS)	–	
PM:GLY	80:20 (wet basis)	Batch	35	–	0.25 (VS)	–	[25]
PM:Microalgae	50:50 (COD basis)	Batch	35	–	0.25 (COD)	–	[47]
PM:Winery WW	15:85 (COD basis)	Batch	35	–	0.35 (COD)	–	[48]
	40:60 (COD ratio)	CSTR	35	0.85 (COD)	0.11 (COD)	–	
PM:Waste sardine oil	1.51:1.66 (VS basis)	CSTR	35–37	3.00 (COD)	0.43 (VS)	$\times 0.95$	[49]
	1.49:2.49 (VS basis)				0.50 (VS)	$\times 1.12$	
	1.46:4:16 (VS basis)				0.46 (VS)	$\times 1.02$	
PM:Sea weed	52:48 (wet basis)	CSTR	38	3.50 (COD)	0.15 (VS)	–	[40]
PM:GLY	20:80 (COD basis)	UASB	n.d	1.70 (COD)	0.15 (COD _{removal})	–	[50]
				5.00 (COD)	0.10 (COD _{removal})	–	

n.d: Non-detailed.

^a ORL organic basis units in brackets.

^b SMP organic basis units in brackets.

^c Multiplication factor to the SMP of mono-digestion considering the same units shown for each study.

^d Specific production expressed in biogas instead of CH_4 .

existent, the waste refining treatment. For instance, the use of GLY as co-substrate can have negative effect due to the presence of methanol and cations (sodium or potassium from the catalyst) [34–36]. Desugared molasses can also be inhibitory because of the high concentrations of sodium and potassium [37]. OMW and wine distillery wastewater inhibitory effect is related to the presence of phenolic compounds [27,38], whereas limonene is the inhibitory compound present in citric waste [39]. Other substrates, like seaweed, can lead to a biogas with high levels of H_2S , making it unsuitable for energy recovery without treatment [40]. Therefore, before the addition of an unknown or insufficiently studied co-substrate, it is highly recommended to perform laboratory experiments to detect the presence of inhibitory compounds, which could lead to a process breakdown or decrease the methane production [25].

Despite these facts, the highest risk of process inhibition when adding C-rich waste as co-substrates is overloading and the resulting digester acidification. In AcoD, the increase of the methane production is mostly linked to the increase of the OLR. Nevertheless, if a certain OLR value is exceeded the process can become unstable, which, if not solved, can lead to digester failure [13,35,41,42]. An AcoD study between PM and grass silage, where three feedstock proportions and four OLR were evaluated, showed that the specific methane production (SMP), at each OLR, was slightly affected by the tested co-substrate proportion [43]. Contrariwise, although higher methane productions were reached, the degradation efficiency and consequently the SMP diminished as the OLR increased. Specifically, as the OLR increased from 1 to 2 and 3 $\text{kg VS m}^{-3} \text{d}^{-1}$ the post-methane potential of the effluent increased from 12% to 22% and 39%, respectively. Comino et al. [44], who treated a mixture of CM and crop silage, evaluated

the effect of three OLR (4.5, 5.2 and 7.8 $\text{kg VS m}^{-3} \text{d}^{-1}$) over AcoD by increasing the co-substrate proportion in the feedstock. The better process performance was reached when the OLR was 5.2 $\text{kg VS m}^{-3} \text{d}^{-1}$, when the crop silage provided 70% of the feedstock VS. However, a further increase of the co-substrate proportion led to the breakdown of the system. Similarly, Astals et al. [13] in a study devoted to PM and GLY co-digestion suggested that the process instability, which was leading the digester to failure when co-substrate supplied about 80% of the organic matter in the influent, was a consequence of (i) the negligible alkalinity of the GLY, which reduced the alkalinity of the mixture and therefore in the digester and (ii) the large amounts of VFA (mainly propionic) generated by GLY degradation.

Although most of the studies have been carried out with one-stage mesophilic continuous stirred tank reactors (CSTR), reported ways to improve AcoD performance or to allow higher OLR are based on thermophilic conditions operation and other reactors configurations. During the period of time examined, some studies focused on comparing mesophilic and thermophilic conditions (Table 2).

The methane yields at thermophilic conditions were slightly higher than at mesophilic conditions. Actually, small-farm reactors may be subjected to temperature fluctuations due to large variations in outdoor temperature. However, the benefits of a proper process temperature show that the biogas production improve and the digester performance presents better stability parameters and economic benefits [20,21]. Regarding digester configuration, Bertin et al. [46], who treated a mixture of CM and cheese whey, reported a 40% methane production increase when a two-stage system (conventional or concentric reactors) was compared to a one-stage system. A more innovative digester configuration is the induced bed reactor suggested by Castrillón et al. [26], which allowed

Table 2
Effect of temperature in manure-based co-digestion systems.

Substrates	Mixture ratio	Digester configuration	T [°C]	OLR [kg ^a m ⁻³ d ^{-1a}]	SMP [m ³ CH ₄ kg ^{-1b}]	Improvement ^c	Reference
CM:FVW	27:73 (wet basis)	CSTR	47	5.67 (VS)	0.32 (VS)	–	[20]
			55	4.66 (VS)	0.38 (VS)	–	
CM:Cheese wey	1:2 (wet basis)	CSTR	25	HRT=5	0.62 ^d	–	[21]
			34	HRT=5	0.90 ^d	–	
CM:OMW	3:1 (n.d)	CSTR	37	5.50 (COD)	0.18 (VS)	× 1.74	[22]
			55	5.50 (COD)	0.21 (VS)	–	
CM:PM:WW	40:30:30 (n.d)	Batch	37	–	0.30 (VS)	–	[23]
			55	–	0.35 (VS)	–	
CM:PM:WW	35:35:30 (n.d)	CSTR	37	1.90 (TOC)	0.21 (VS)	× 1.68	[24]
			55	1.76 (TOC)	0.28 (VS)	× 1.72	
CM:Energy crops	50:50 (VS basis)	CSTR	37	2.00 (VS)	0.21 (VS)	× 1.60	[24]
			37	4.00 (VS)	0.17 (VS)	× 1.66	
			55	2.00 (VS)	0.22 (VS)	Thermophilic mono-digestion failed	
			55	4.00 (VS)	0.23 (VS)	Thermophilic mono-digestion failed	
CM:Energy crops: FVW	50:25:25 (VS basis)	CSTR	37	2.00 (VS)	0.25 (VS)	× 1.91	[24]
			37	4.00 (VS)	0.25 (VS)	× 2.44	
			55	2.00 (VS)	0.29 (VS)	Thermophilic mono-digestion failed	
			55	4.00 (VS)	0.28 (VS)	Thermophilic mono-digestion failed	

n.d: Non-detailed.

^a ORL organic basis units in brackets.

^b SMP organic basis units in brackets.

^c Multiplication factor to the SMP of mono-digestion considering the same units shown for each study.

^d Units m³ CH₄ m⁻³ d⁻¹.

higher OLR and organic matter removals than the conventional CSTR when co-digesting CM and GLY.

2.2. Digestate quality for agricultural use

From an environmental and economic point of view, applying AD digestates, a mixture of partially degraded organic matter, anaerobic biomass and inorganic matter, as organic fertilizer or soil conditioner seems to be the best option for its recycling, since it contains considerable amount of nutrients [13,51]. Furthermore, in some cases, further income can be gained from digestate sale [10]. Digestate properties are conditioned by the feedstock, the development of the anaerobic process in the digester, and the digestate post-treatment [51,52]. The main risk over digestate quality when a co-substrate is added is the production of unstable digestates (remaining a high concentration of easy biodegradable organic matter), which may exert negative impacts on organic matter mineralization and nutrient turn-over in the plant-soil system [51,53]. This is mainly because of the prevalence of the efficiency criteria for biogas production over digestate stability, a fact that can lead to a shortening of the residence time of the material in the digester and, as a result, the produced digestate might not be completely exhausted in terms of labile organic matter [4,13,54,55]. Despite this fact, the use of digestates derived from manure anaerobic digestion into the soil may depend on its (i) chemical properties, (ii) stability, and (iii) hygienization.

From an agricultural point of view, parameters like pH, salinity, nutrients and heavy metals are the chemical properties more important to consider. Alburquerque et al. [54], who evaluated 12 AcoD digestates (6 PM and 6 CM), found that PM digestates have higher conductivity values than CM digestates, with Cl, Na and Ca the ions having a higher concentration. It should be noted that high doses or continued applications of high salinity digestates can lead to an excessive salt accumulation in soil, which might inhibit

plant growth [54,56]. Regarding heavy metals, Alburquerque et al. [54] reported high concentrations of Cu and Zn in CM and PM, while Demirel et al. [57] reported high concentrations of Zn, Cu and Ni in poultry manure. Both authors attributed the presence of these heavy metals to the commercial feedstuffs, which contain several chemicals to promote optimum growth rates and to prevent livestock diseases. As a general trend, although it depends on the co-substrate characterization, using agro-industrial wastes as co-substrates may help to reduce the concentration of some elements (especially heavy metals) [57,58]. Additionally, Alburquerque et al. [59] evaluated through two horticultural crops (watermelon and cauliflower) the fertilizing capacity of a digestate obtained from an full-scale AcoD plant, which treats PM together with sludge from a slaughterhouse and biodiesel wastewater. The study showed that the digestate had a positive effect on watermelon (grown in summer), but very little effect on cauliflower (grown in winter). This may be related to the winter conditions (rain favoring nutrient leaching and lower nitrification rate) as well as the larger crop cycle of cauliflower [59].

Digestate stability is a factor of utmost importance, since using unstable digestates can cause N-immobilization and/or oxygen exhaustion because of an excessive increase in soil microbial activity [51,59]. Nonetheless, there is little agreement about which parameter/s and threshold limit/s should be used to assess digestate stability. Although many parameters have been suggested as indicators of the digestate stability, for semi-solid wastes, respirometric indexes seem more adequate [13]. Specifically, digestate stability has been evaluated through 5-day biochemical oxygen demand (BOD_{5d}) [4,13,51], dynamic respirometric index [60,61], and post-methanization potential [43,44,60,62,63]. However, these techniques are relatively time consuming and therefore several efforts have been made to develop faster procedures to assess digestate stability. In this regard, Gómez et al. [64] used derivative thermogravimetry profiles, while Tambone et al. [65] used solid-state ¹³C cross-polarization magic angle spinning nuclear magnetic resonance spectroscopy. Nonetheless, at present,

digestate stability should be assessed through the combination of several parameters [4,51].

Finally, because animal manures are known to contain pathogens, the digestate must have a low occurrence of pathogens prior to land application. Otherwise, new ways of transmission of pathogens between people and animals could be established [66]. In this vein, thermophilic digestates and some pre-treatments are known to generate digestates that fulfill the American and the European legislation for land application, while a post-treatment (pasteurization, composting, etc.) is required for mesophilic digestates prior to its use in land [4,31,67].

3. Sewage sludge as a main substrate

Sewage sludge ranks as the second main substrate for AcoD. Historically, AcoD between SS and OFMSW is the most reported co-digestion research. However, between 2010 and 2013 the publications dealing with fats, oils and greases (FOG) as SS co-substrate have steeply increased, amounting to 26% of the publications. Within the same period, other co-substrates such as fruit and vegetable waste (FVW), SHW, GLY, and algae have also been broadly reported.

The low organic load of the SS together with the non-used capacity of the wastewater treatment plants (WWTP) digesters, frequently as much as 30%, is the main driving force behind SS co-digestion [68,69]. SS is characterized by relatively low C/N ratio and high buffer capacity [70,71]. Therefore, it is able to stand co-substrates with high amounts of easily biodegradable organic matter and with low alkalinity values. Moreover, in many cases, SS co-digestion can also lead to the dilution of some undesired compounds present in SS such as heavy metals, pharmaceuticals and/or pathogens [72,73]. Taking into account these facts and considering that the transport cost is the first selection criteria, both OFMSW, produced in every municipality, and FOG, discharged by the grit chamber at the WWTP, are very convenient co-substrate for SS. It goes without saying that any agro-industrial waste generated in the industrial parks near the WWTP would be as convenient as the other two.

3.1. Sewage sludge co-digestion with biowaste

In the present section, the term biowaste includes OFMSW (all qualities), food waste, market waste and FVW. Besides the feedstock composition, a wide range of results in the literature (normally successful) concerning SS and biowaste co-digestion are consequences of several factors such as SS composition (primary, secondary or mixed), OLR, digester configuration, temperature range or mixing conditions [3] (Table 3).

Biowaste represents a highly biodegradable co-substrate, which, until a certain threshold limit is surpassed, improves the biogas production of the SS digesters just by increasing the OLR. In this matter, Krupp et al. [69], who evaluated the economic and environmental suitability of using OFMSW as co-substrate in two German WWTP, concluded that using OFMSW as SS co-substrate was the most advantageous solution when compared to OFMSW composting and mono-digestion. Although this particular mixture is a good cross-sectorial opportunity, as shown in a review of benefits and constraints carried out by Iacovidou et al. [72], SS: OFMSW AcoD is not so easy in practice. Iacovidou et al. [72] stated that it is generally faced with (i) complex and unclear regulatory framework, (ii) sorting pre-treatment of the OFMSW prior its AD, (iii) composition variability and seasonality, and (iv) possible inhibitions caused by VFA accumulation, light metals (especially with FVW), LCFA (adding biowaste rich in lipids) and/or NH₃ (adding biowaste rich in proteins). Moreover, Pahl et al. [89]

reported an accumulation of heavy metals (specially Zn, Pb and Ni) and improper materials in the WWTP digestate when mechanically-sorted OFMSW was introduced as co-substrate. However, these problems mainly related to OFMSW sorting and pre-treatment do not appear in pilot and even less in lab-scale conditions, where most of the studies have been carried out.

The effect of the OLR, ranging from 1.2 to 8.0 kg VS m⁻³ d⁻¹, over process performance and stability was evaluated in a mesophilic pilot plant that co-digested WAS and biowaste (mix of OFMSW and FVW) by Liu et al. [90]. Although the process showed a maximum biogas production when the digester was operated at 8.0 kg VS m⁻³ d⁻¹, the high VFA level together with the reduction of the biogas yield (from 0.73 to 0.62 m³ kg⁻¹ VS) indicated a higher risk of acidification. In the case of SS and FVW (pear residues) AcoD, Arhoun et al. (2013) evaluated the influence of two feeding strategies: discontinuous (once per day) and pseudo-continuous, where liquid and pulp fed followed different patterns. Although the biogas yield remained constant when the OLR was changed (about 0.44 m³ kg⁻¹ VS), the pseudo-continuous scheme allowed to achieve higher OLR than the discontinuous one (10.5 and 6.0 kg VS m⁻³ d⁻¹, respectively). Even though most WWTP digesters are operated under mesophilic conditions, the need to enhance the biogas yield and sludge hygienization has raised interest in thermophilic conditions, which is not without its disadvantages [91]. However, OFMSW industrial digesters are almost equally distributed between mesophilic and thermophilic conditions [92]. Alternatively, temperature phased anaerobic digestion has been studied by a number of researchers in order to overcome thermophilic AD drawbacks [91]. For instance, Cavinato et al. [76] evaluated, in a pilot and full-scale plant, the feasibility of mesophilic and thermophilic conditions over a mixture of WAS and OFMSW (a mix of separately and source collected OFMSW). The authors reported that thermophilic conditions improved the biogas yield around 45–50%, which allowed the thermophilic process to obtain the higher heat requirements. Similarly, Kim et al. [74], who co-digested SS and separately collected OFMSW, reported an increase of 0.2 m³ CH₄ kg⁻¹ VS when a thermophilic–mesophilic temperature phased system was compared with a mesophilic–mesophilic one.

3.2. Sewage sludge co-digestion with fats, oils and greases

Due to its high methane potential 0.7–1.1 m³ CH₄ kg⁻¹ VS, FOG is a very interesting co-substrate for SS AcoD, even more when it is an in-house waste. Nonetheless, FOG dosing rate must be limited in order to avoid high concentration of LCFA (result of lipid degradation) in the digester, a potential inhibitor of the methanogenic activity [93,94]. Moreover, FOG has been related with other operational problems like clogging in the liquid or gas systems, foaming and biomass flotation related to adsorption of lipids onto biomass [95,96]. Despite these facts, a high number of successful experiences have been reported when SS and FOG, from different origins, have been co-digested (Table 3). Specifically, the FOG comes mainly from two sources: FOG generated in the same WWTP and FOG from industrial processes (including the treatment plant). In WWTPs, FOG represents 25–40% of the wastewater total chemical oxygen demand (COD) and it is usually removed (50–90%) prior biological treatments [77,97,98]. Additionally, its utilization as a co-substrate represents saving the cost of treating the residue outside the plant.

Fig. 4 summarizes publications dealing with mesophilic continuous digesters treating SS or WAS together with FOG. Regarding the FOG obtained from a WWTP, Noutsopoulos et al. [77] recorded a 285% increase of the biogas production when compared to a 40% SS and 60% FOG mixture on VS basis (2.1 kg VS_{FOG} m⁻³ d⁻¹) with the SS mono-digester, which had an OLR of 1.9 kg VS m⁻³ d⁻¹. However,

Table 3

Summary of SS co-digestion studies with biowaste, FOG, SHW and GLY in lab-scale and pilot-scale digesters.

Substrates	Mixture ratio	Digester configuration	T (°C)	OLR [kg ^a m ⁻³ d ⁻¹]	SMP [m ³ CH ₄ kg ^{-1b}]	(a) Improvement ^c (b) Inhibition	Reference
<i>Sewage sludge co-digestion with biowaste</i>							
SS:FW	60:40 (VS basis)	SBR -Temperature Phased	35+55	3.50 (VS)	0.18 (VS)	(a) – (b) –	[74]
		SBR -Temperature Phased	55+35	6.10 (VS)	0.20 (VS)	(a) – (b) –	
WAS:Biowaste	1: 1 (n.d)	CSTR	35	4.80 (VS)	0.42 (VS)	(a) – (b) High VFA and poorer yields at ORL > 4.8	[75]
SS:FW	0.9: 1 (VS basis)	CSTR	35	7.20 (VS)	0.33 (VS)	(a) × 1.72 (b) NH ₄ ⁺ –N (from SS) and Na ⁺ (from FW)	[73]
WAS:OFMSW	n.d	n.d	37	1.60 (VS)	0.21 (VS)	(a) × 2.28 (b) –	[76]
	n.d		55	2.21 (VS)	0.35 (VS)	(a) – (b) –	
<i>Sewage sludge co-digestion with FOG and other agro-industrial wastes</i>							
SS:FOG	40:60 (VS basis)	CSTR	35	3.50 (VS)	0.49 (VS)	(a) × 1.67 (b) LCFA Substrate transport limitation Digester foaming	[77]
SS:FOG	36.4:63.6 (COD basis)	CSTR	35	11 (COD)	0.31 (COD)	(a) – (b) –	[78]
WAS:FOG	48:52 (VS basis)	CSTR	36	1.2 (VS)	0.55 (VS)	(a) × 2.07 (b) 74% (VS basis)	[79]
SS:FOG	77:23 (VS basis)	CSTR	35	1.6 (VS)	0.37 (VS)	(a) × 1.48 (b) –	[71]
SS:Grease trap waste	77:23 (VS basis)	CSTR	36	1.58 (VS)	0.63 (VS)	(a) × 1.27 (b) LCFA accumulation at 30% FOG (VS basis)	[80]
WAS:FOG	64:36 (VS basis)	CSTR	37	2.34 (VS)	0.60 (VS)	(a) × 2.37 (b) Acidification at 75% FOG (VS basis)	[81]
SS:FOG	99.8:0.2 (volume basis)	CSTR	34	0.77 (VS)	0.30 (VS)	(a) – (b) A continuous decrease in the HRT adsorption of lipids onto active sludge	[82]
SS:used oil	80.6:19.4 (VS basis)	CSTR	38	0.91 (VS)	0.47 (VS)	(a) – (b) Inhibition at 53% used oil (VS basis)	[83]
WAS:FOG	34.5:65.5 (VS basis)	CSTR	37	2.16 (VS)	0.75 (VS)	(a) × 4.18 (b) Inhibition at 83.5% FOG (VS basis)	[84]
SS:SHW	95:5 (wet basis)	CSTR	37	2.68 (VS)	0.62 (VS)	(a) × 2.65 (b) High NH ₃ concentration at 7.5% SHW (wet basis) Accumulation and intense foaming at 10% SHW (wet basis) LCFA	[85]
SS:SHW	7:1 (wet basis)	CSTR	35	2.8 (VS)	0.43 (VS)	(a) – (b) At 14 days-HRT, SMP decreased indicating too high OLR	[86]
SS:GLY	99:1 (volume basis)	CSTR	35	HRT=23–25 d	0.78 ^d	(a) × 2.13 (b) VFA accumulation at 3% GLY (volume basis)	[87]
SS:GLY	77: 23 (VS basis)	CSTR	36	1.04 (VS)	0.86 (VS)	(a) × 1.83 (b) 31% GLY (VS basis) Increase of VFA and decrease in % CH ₄	[88]

n.d: Non-detailed.

^a ORL organic basis units in brackets.^b SMP organic basis units in brackets.^c Multiplication factor to the SMP of mono-digestion considering the same units shown for each study.^d units m³ CH₄ m⁻³ d⁻¹.

the co-digestion process failed when the digester OLR was further increased and the FOG organic loading rate (OLR_{FOG}) was 2.5 kg VS_{FOG} m⁻³ d⁻¹. Nonetheless, Alanya et al. [78], who co-digested SS with FOG from the surface of the primary settlers and from the scum concentration tank, obtained a 410% increase of the biogas production with a similar FOG load (~2.4 kg VS_{FOG} m⁻³ d⁻¹). The OLR_{FOG} applied by Noutsopoulos et al. [77] and Alanya et al. [78] was much higher than those utilized by Davidsson et al. [99] (0.7 VS_{FOG} m⁻³ d⁻¹) and Silvestre et al. [71] (0.6 kg VS_{FOG} m⁻³ d⁻¹), who also recorded a significant increase of the biogas production (150% and 210% respectively), when compared with the SS mono-digester. The differences between publications can be attributed, among others, to the characteristics of the feedstock and anaerobic biomass, the digester operational conditions and the adaptation periods. Other studies have evaluated the co-digestion between SS or WAS with FOG coming from

other origins. For instance, Razaviarani et al. [80], who studied in pilot-scale the co-digestion between SS and restaurant grease trap waste, obtained a 67% greater biogas production, with respect to the mono-digestion, when dosing 0.36 kg VS_{FOG} m⁻³ d⁻¹. However, when the grease trap waste dose was increased to 0.48 kg VS_{FOG} m⁻³ d⁻¹, a marked decline in biogas production was observed. Pastor et al. [83] reported, after testing several operational conditions for SS and used oil co-digestion, an optimum FOG dosing rate of 0.48 kg VS_{FOG} m⁻³ d⁻¹. Moreover, the authors also observed that an excessive addition of used oil (1% in wet weigh basis) led to a significant increase of the H₂S concentration in the biogas.

Although the FOG organic loading rate threshold is not clear enough, safe co-digestion performance and high biogas productions have been recorded for OLR_{FOG} up to 0.8 kg VS_{FOG} m⁻³ d⁻¹. Nonetheless, when FOG was co-digested with WAS instead of SS,

the OLR_{FOG} limit seems to be higher (Fig. 4). This observation may be explained by the lower lipids concentration and/or the lower biodegradability of the WAS when compared with SS. Actually, Wan et al. [81] recorded a 125% enhancement of the biogas production when WAS and FOG ($1.5 \text{ kg VS}_{FOG} \text{ m}^{-3} \text{ d}^{-1}$) from receiving facility were co-digested. The aforementioned OLR_{FOG} threshold is in agreement with the value reported by Wang et al. [84], who recorded the maximum biogas production at a FOG load of $1.5 \text{ kg VS}_{FOG} \text{ m}^{-3} \text{ d}^{-1}$ when thickened WAS and grease interceptor waste from a food service establishment were co-digested. Contrariwise, the threshold value suggested by Girault et al. [79], who co-digested WAS and FOG from a dissolved air flotation unit that processes wastewater from a pork processing industry, is similar to the proposed for SS ($0.62 \text{ kg VS}_{FOG} \text{ m}^{-3} \text{ d}^{-1}$). A way to improve the FOG load into a digester is, as suggested by Donoso-Bravo and Fdz-Polanco [102], the application of enzyme-lipase, which is capable of catalyzing the LCFA degradation. Specifically, the authors observed a significant increase of the production rate and the total methane production when lipase doses between 0.33–0.83% on volume basis were applied.

3.3. Sewage sludge co-digestion with other organic wastes

Even though most publications have applied FOG or biowaste as sewage sludge co-substrate, other publications have evaluated the potential of other co-substrates such as algae (micro and macro) or agro-industrial waste (i.e. GLY, SHW) among others (Table 3).

Algae are particularly suitable for methane production, because of their large availability in nature, low impact in food markets and low lignin content [103–105]. For instance, Vivekanand et al. [106] identified *Saccharina latissimais*, a fast-growing brown seaweed with a SMP of $0.22 \text{ m}^3 \text{ kg}^{-1} \text{ VS}$, as a marine energy crop. However, the potential of the algae lay in the fact that their growth can be used as a treatment for wastewaters nutrient removal. In this matter, Rusten and Sahu [107] cultivated *Chlorella sp.* microalgae to treat AD reject water and afterwards use it as a sewage sludge co-substrate. *Chlorella sp.* showed nitrogen removal between $0.08\text{--}0.09 \text{ kg N kg}^{-1} \text{ TSS}$ and SMP around $0.25 \text{ m}^3 \text{ kg}^{-1} \text{ VS}$. Moreover, this approach may also reduce the impact of the returning side-streams to the head of the WWTP, which account for 20–30% of the WWTP nitrogen load [107]. Similarly, Yuan et al. [108] evaluated the growth of two species of microalgae (*Chlorella sp.* and *Spirulina platensis*) when treating both rejected water and a mixture of rejected water and nitrified wastewater. *Chlorella sp.* was able to growth in both mediums, while *S. platensis* only

grew well in the mixed effluent, possibly due to the toxicants and/or the lack of critical growth substrates. Although the SMP was not reported, both microalgae showed higher VS removal efficiencies than WAS. Higher VS removal efficiencies, compared to WAS mono-digestion, were also obtained when both microalgae were co-digested with WAS. However, only *S. platensis* AcoD digestate showed a better dewaterability than WAS mono-digestion, while *Chlorella sp.* worsened it. These later results are not in agreement with those obtained by Wang et al. [109], who evaluated the anaerobic co-digestion between *Chlorella sp.* and WAS. Wang et al. [109] also observed that WAS and *Chlorella sp.* AcoD was a feasible approach to improve digester biogas production; however, they recorded that the digestate from the co-digestion had a better dewaterability than WAS digestate.

Regarding macroalgae, Costa et al. [110] determined the SMP of several macroalgae, i.e. *Ulva sp.*, *Gracilaria sp.* and *Gracilaria vermiculophylla*. The highest SMP was obtained for *Ulva sp.* ($0.20 \text{ m}^3 \text{ kg}^{-1} \text{ VS}$), while *Gracilaria sp.* and *G. vermiculophylla* showed SMP of 0.18 and $0.15 \text{ m}^3 \text{ kg}^{-1} \text{ VS}$, respectively. The authors also reported that *Ulva sp.* presented the highest hydrolysis rate. According to the authors, *Ulva sp.* is characterized by a low content of lignin, a thinner and simpler morphological structure, and a large surface area, which make it easier to digest when compared to the other tested macroalgae. Afterwards, Costa et al. [110] performed co-digestion experiments between WAS and *Ulva sp.*, which presented a remarkable increase of the methane production without decreasing the overall biodegradability. In a subsequent study, Sode et al. [111] successfully cultivated *Ulva lactuca* to recover N and P from sewage sludge AD reject water, which is due to its higher methane potential and kinetics more economically attractive than the other macroalgae [110].

Several agro-industrial wastes, not classified as biowaste, have also been successfully tested as SS co-substrate. Among them, GLY and SHW stand as the most interesting ones. As described in Section 2.1, the highest risk when using GLY as co-substrate is overloading and the resulting digester acidification. In this vein, the threshold value reported when co-digesting SS and GLY, about 1% [87,88], is lower than that reported for manure-based digesters, which range between 3% and –6% [4,13,35,112]. Although there is no specific data, the difference may be related to several factors, for instance: main substrate biodegradability and C/N ratio, biomass concentration, distribution and adaptation, digester performance and/or inhibitory compounds present in GLY [4,87,88]. The AcoD between SS and sterilized SHW have been studied by Luste and Luostarinen [86] and Pitk et al. [85]. Both publications, where different mixing ratios and OLR were tested, stated the

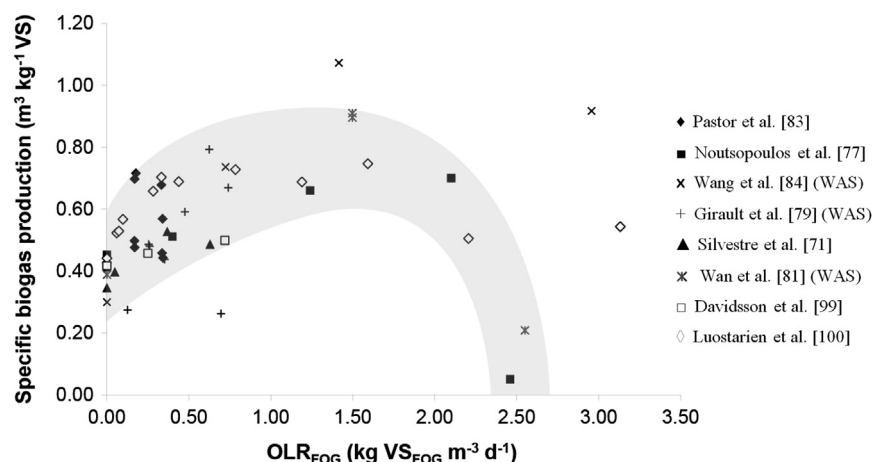


Fig. 4. Specific biogas production depending on the OLR_{FOG} co-digested with SS or WAS in mesophilic under continuous or semi-continuous operation. Including reference [100].

sterilized SHW is an ideal waste to improve SS AD performance; however, when a certain dose was exceeded the process failed due to ammonia and/or LCFA inhibition. As far as we know, other tested co-substrates are landfill leachates [68,83], sugar beet pulp lixiviates [113], corn straw [114], shredded grass [115], tannery solid waste [116] and pulp and paper mill wastewater [117].

3.4. Sewage sludge co-digestion in full-scale plants

Full-scale SS co-digestion practices are not as reported as would be expected. This is mainly because of the low interest of the industrial sector to publish their results in the scientific literature. Table 4 summarizes the reported full-scale SS co-digestion experiences, where the mixture between SS and bio-waste is the most reported one.

In the WWTP of Frutigen (Switzerland, 8000 p.e.), Edelmann et al. [118] increased the biogas production to 25% when adding in a mixed SS digester of about 1 m³ per day of separately-collected OFMSW (supermarket and hospital organic waste). The organic waste, which was size-reduced prior to digestion, increased to 20% of the digester OLR without any impact on the working conditions or the operation of the plant. Similarly, Park et al. [121] co-digested chopped FVW from food stores with sewage sludge at the WWTP of Prince George (Canada, 115,000 p.e.). The biogas production was increased between 8% and –17% by adding 280–420 kg per day. The only reported mechanical problem was the clogging of the hose that connected the chopper pump to the sludge recirculation line. However, more worrisome was the increase of impurities in the digestate, since it may make difficult its land application. Bolzonella et al. [122] reported the successful implementation of the WAS – OFMSW co-digestion at two Italian WWTP, Viareggio (100,000 p.e.) and Treviso (70,000 p.e.). At Viareggio, the addition of 3–5 t of source-collected OFMSW increased 1.0–1.2 kg VS m⁻³ d⁻¹ the OLR and 50% the biogas production, while at Treviso the addition of 8–9 t of separately-collected OFMSW allowed to increase the biogas production by 240%. In a subsequent study at the Treviso WWTP, Cavinato et al. [76] proved that changing the co-digester operational conditions from mesophilic to thermophilic led to improvements of the biogas production by around 40%. Another example of SS – OFMSW co-digestion is reported by Zupancic et al. at a 50,000 p.e. WWTP located in Velenje (Slovenia), where an 80% increase of the biogas production was reached. Finally, Dereli et al. [123] and Krupp et al. [69] have shown the economic advantages of this approach when compared to a separate treatment solution for each kind of waste.

The mixture of SS and FOG has also been considerably implemented at full-scale. However, unlike lab-scale publications,

the experiences applying used oils (from industries or restaurants) have been more reported than the ones using FOG from their own grit chamber. At the WWTP of La Pobla de Farnals (Spain, ~200,000 p.e.), Pastor et al. [83] increased by about 23% the biogas production when adding between 0.5% and 1% (weight-basis) of used oil in the digester feedstock. Nonetheless, the authors recorded a significant increase of the H₂S concentration in the biogas when feeding 1% or higher doses of used oil. Other examples of the full-scale implementation of this mixture are the WWTP of Watsonville (United States, ~60,000 p.e.), which have been co-digesting used oil with SS for more than four years, the WWTP of Riverside (United States, ~60,000 p.e.) or the WWTP of Annacis Island in Vancouver (Canada, > 1,000,000 p.e.) [101].

4. Biowaste as a main substrate

Biowaste, largely OFMSW (all qualities), as a main substrate has not been as studied as manures or SS (Fig. 2). That may be a consequence of several factors: the high biogas potential of this kind of waste, the lower number of plants and their location, but above all the presence of undesired compounds. The latter makes that the digestates, followed or not by composting, able to be used in restricted applications (e.g., land reclamation, landfill daily cover, etc.) or even dumped in landfills. Apart from these facts, the OFMSW has typically been used as co-substrate for SS because, in many towns, WWTP were already equipped with anaerobic digesters. Indeed, co-digestion between SS and OFMSW has been reflected as a way to reduce significantly the treatment costs of both wastes [69,124].

All the publications found in this review, where biowaste was used as main substrate, have been carried out in lab-scale digesters and 80% of them were done in wet conditions (TS concentration below 15%). The papers have focused on process parameters, i.e. mixing ratio, maximum OLR or interactions between substrates, as well as searching for new co-substrates such as in-plant streams [125], paper waste [126,127] or distiller's grain [128]. In this regard, Nayono et al. [125] utilized press water from a composting process as co-substrate, which is a great opportunity to integrate waste streams in those mechanical-biological treatment plants that have both processes. Nayono et al. [125] observed how the biogas production increased linearly as the OLR increased from 12 to 20 kg COD m⁻³ d⁻¹ (feeding 5 days a week), where the maximum OLR for safety operation was recorded. Specifically, over 20 kg COD m⁻³ d⁻¹, the process became very unstable due to the accumulation of VFA, especially propionate. Similarly, Kim and Oh [126], who co-digested biowaste and paper waste in a continuous dry-digester, searched for the best process performance by changing the feedstock solid

Table 4
Summary of SS co-digestion studies in full-scale plants.

Substrates	Mixture ratio	Digester configuration	T (°C)	OLR [kg ^a m ⁻³ d ⁻¹]	SMP [m ³ CH ₄ kg ^{-1b}]	Improvement ^c	Reference
SS:FVW	82.3:17.7 (wet basis)	n.d	M	HRT=20d	0.57 ^d (RAW)	× 1.03	[118]
WAS:OFMSW	84.3:15.7 (VS basis)	n.d	M	1.21 (VS)	0.17 (VS)	× 1.21	[119]
	59:41 (VS basis)	n.d	36.3	0.78 (VS)	0.28 (VS)	× 1.97	
SS:OFMSW	80:20 (VSS basis)	CSTR	M	1.00 (VSS)	0.60 ^d (VSS)	× 1.54	[120]
WAS:FVW	n.d	2 digesters	36	n.d	n.d	× 1.08–1.17	[121]
WAS:OFMSW	n.d	n.d	37	1.62 (VS)	0.21 (VS)	–	[76]
			55	1.28 (VS)	0.33 (VS)	–	
SS:used oil	94:6 (VS basis)	CSTR	M	HRT=57d	0.63 ^d (VS)	× 1.24	[83]

n.d: Non-detailed.

M: Digester operated at mesophilic conditions.

^a ORL organic basis units in brackets.

^b SMP organic basis units in brackets.

^c Multiplication factor to the SMP of mono-digestion considering the same units shown for each study.

^d Specific production expressed in biogas instead of CH₄.

concentration and the digester HRT. The higher biogas production was obtained when the feedstock had a TS concentration of 40% and the digester HRT was 40 day. As for Nayano et al. [125], when the OLR was further increased (TS concentration was increased up to 50%), a drastic decrease of process performance was recorded [126]. It is well known that VFA inhibition, due to digester overload, is a significant constrain during OFMSW mono- and co-digestion. However, the threshold limit varies considerably depending on the process configuration. In this way, percolation systems stand higher VFA concentration than continuous digesters; whereas within the continuous digesters, dry systems allow higher VFA concentration than wet ones [125,128–130]. Besides, depending on the co-substrate used and process performance, ammonia inhibition can also occur [126,130,131].

Regarding co-digestion synergisms, Zhang et al. [132], who co-digested biowaste with the solid and liquid fraction of the piggery wastewater, attributed the improved biogas yield and higher digester stability to the trace elements supplemented by the wastewater rather than to the C/N ratio balance. The authors stated that the wastewater procured some trace elements (e.g., Co, Ni, Mo, Fe), which biowaste lacked, which improved biomass enzymatic activity and therefore process performance. Xu and Li [129], in a study where biowaste and corn stover were co-digested, evaluated the contribution of each major component (xylan, cellulose, starch, protein and lipids) to the biogas production and how their degradation is affected by the co-substrate addition. As a general trend, starch and protein presented higher degradation extent than lipids, cellulose and xylan. However, the degradation of the major components of corn stover (cellulose and xylan) seemed inhibited when the mixture was rich in biowaste. The authors suggested that the products of the starch hydrolysis from biowaste inhibited corn stover degradation. Likewise, Ponsa et al. [133] evaluated co-substrates for biowastes, i.e. vegetable oil, animal fats, cellulose and peptone (protein). Although all co-substrates led to operative improvements in process parameters (kinetic and extent), the authors concluded that vegetable oil (FOG waste) was the most suitable co-substrate for biowaste.

5. Pre-treatments as option to improve AcoD performance

Pre-treatments applied to AcoD were rarely described in literature until 2011, when the production of papers related to this topic steeply increased. This boom is partly because, in some regions, easy biodegradable co-substrates are already being used or not available and consequently the utilization of more complex organic sources as co-substrates have been considered. Most of the research has been devoted to mechanical pre-treatments (including ultrasound) with a 33% of occurrence, followed by thermal pre-treatments (24%) and chemical pre-treatments (21%). The main goal of the pre-treatment in AcoD is the same as that pursued in AD practice, i.e. to render a more biodegradable (extent and kinetics) substrate to the digestion process so that methane production is increased. In AcoD, pre-treatments have been mostly applied over one of the co-substrates, obviously the one with a poorer biodegradability, rather than over the whole mixture. The reason is that to treat the mixture instead of a single substrate increases, due to the larger volume to be treated, the capital and, especially, the operating expenses.

Considering the results reported for ultrasound pre-treatment (US), as a general trend, the slight increase of the SMP is far from compensating for the electricity cost of the pre-treatment. Li et al. [134] who evaluated different US conditions over FOG (5300–36,000 kJ kg⁻¹ TS) and OFMSW (1700–14,000 kJ kg⁻¹ TS), reported a slight increase of the OFMSW SMP and no effect over FOG SMP. Moreover, sonicated FOG displayed a larger lag phase in the

accumulated methane curve. Cesaro et al. [135] recorded a 24% increase on the biogas production when 90,000 kJ kg⁻¹ TS were dosed to a mixture of SS and OFMSW. Luste et al. [31], who pre-treated a mixture of CM and SHW, reported an 11% increase of the SMP when applying either 1000 or 6000 kJ kg⁻¹ TS to the mixture. Similarly, a small increase of the SMP was recorded by Marañón et al. [42] when dosing 7500 kJ kg⁻¹ TS to a mixture of CM and GLY. Higher improvements were obtained by Castrillon et al. [36] who reported a 120% biogas production increase when screened cattle manure was ultrasonicated (29,000 kJ kg⁻¹ TS), and Sri Bala Kameswari et al. [136], who reached a 50% biogas generation increase when a mixture of sewage sludge (from tannery wastewater) and fleshing was sonicated.

Among all thermal pre-treatment options, steam explosion is the one that has shown better results at increasing the SMP of different lignocellulosic wastes [137–140]. Steam explosion involves high temperature (between 150 and 250 °C) for few seconds up to several minutes followed by a rapid pressure drop (explosion) [140,141]. During the process, the lignocellulosic structure is opened up which causes a reduction of the sample crystallinity, a release of soluble compounds and an improvement of the accessible surface area [138,141,142]. Vivekanand et al. [140], who steam exploded birch (*Betula pubescens*) under 13 different conditions, recorded the highest SMP (1.8 times higher than the un-treated birch) when the sample was pre-treated at 220 °C for 10 min or at 230 °C for 5 min. The authors stated that the improvement was due to the degradation of xylan and formation of pseudo-lignin during the pre-treatment. Similar optimum conditions were reported by Horn et al. [138], when pre-treating *Salix* chips. The authors observed how the release of glucose and the SMP increased as the treatment intensity increased. A maximum was reached when the sample was pre-treated at 210 °C for 10 min and obtaining similar values at harsher conditions [138]. Estevez et al. [139], who co-digested CM and steam exploded *Salix* (10 min at 210 °C) at different C/N ratios, reported a 50% biogas production improvement due to the *Salix* pre-treatment. Using a marine seaweed (*Saccharina latissimais*), Vivekanand et al. [106] were able to improve the SMP up to 20% when applying 130 °C and 160 °C, respectively, for 10 min. It is clear that steam explosion is a feasible pre-treatment to increase biogas production of some waste and at the same time moderate consumption of energy are claimed [141]. Actually, Shafiei et al. [143] demonstrated through a techno-economic analysis the profitability and performance of steam explosion pre-treatment for wheat straw and paper tube residuals. The authors concluded that applying steam explosion resulted in 13% higher total capital investment; however, a 36% reduction of the manufacturing cost was estimated due to the increased methane production.

High-temperature pre-treatments (150–220 °C), without explosion, have been frequently associated with reductions of the methane potential due to the formation of refractory compounds to AD, e.g., via Maillard reactions [144,145]. For instance, Carrere et al. [146] recorded a 17% drop in the SMP when treating at 170 °C for 30 min a mixture of fatty wastewater and waste activated sludge (WAS). The authors corroborated that the pre-treatment of the fatty wastewater led to the formation of recalcitrant compounds, while the pre-treated SS resulted in a higher SMP. Similar results were obtained by Cuetos et al. [67], when the mono-digestion or co-digestion with OFMSW of pre-treated SHW (for 20 min at 133 °C, > 3 bar) was attempted. On the contrary, Zhou et al. [147], who pre-treated a mixture of SS and OFMSW at 170 °C for 60 min, did not observe any impact on biogas production, but improvements in stability, dewaterability and kinetics.

Low-temperature pre-treatment (60–90 °C) has shown positive results, although normally larger contact times are required. Luste et al. [31], who hygienized (70 °C, 60 min) a mixture of CM and SHW, reported when compared with the untreated sample a 20% and 8% increase of the SMP in batch and continuous experiments,

respectively. An interesting case of study is the carried out by Blank and Hoffmann [148] in a Germany full-scale plant treating SS and OFMSW. The authors decided, after comparing low-temperature (36, 42 and 52 °C) and ultrasound pre-treatment, to install a hydrolytic digester (42 °C and 23 h) prior to AD. The biogas production of the AcoD process was estimated to increase up to 13%. Higher biogas production improvements have been reported when low-temperature have been coupled with alkaline pre-treatment. For instance, Carrere et al. [146], who pre-treated a mixture of fatty wastewater and WAS at 80 °C together with 0.14 kg KOH kg⁻¹ VS (pH=8), recorded a 58% increase on the biogas production. Actually, when dealing with fatty streams, such as SHW or FOG, alkaline pre-treatment helps with the saponification of the lipids as well as promotes the lipid bioavailability [149]. Nonetheless, alkaline pre-treatment has problems related with the chemicals purchasing cost and the cation (sodium or potassium) level in the digester, which can lead to process inhibition [146,150]. Therefore, chemical dosage should be controlled in order to avoid process inhibition.

Finally, some research has dealt with the introduction of biomass with high cellulolytic activity, like rumen microbes, to improve cellulose, hemicellulose and lignin removal efficiency [151]. A study carried out by Chen et al. [152], where corn stalk and vermicompost were co-digested, showed that the high chitinase concentration in vermicompost contributed to the further degradation of chitin and therefore to the enhancement of methane production. The larger destruction of crystalline cellulose present in the corn stalk was confirmed through X-ray diffractometry.

6. Microbial dynamics

During later years, the development of molecular techniques based on 16S rRNA sequences, such as FISH, PCR, DGGE or T-RFLP, have led to an increase of the number of papers dealing with the study of the microbial community. This is mainly due to the reduction of the investment (equipment) and operating (fungible and technician) costs, the increase of the methods reproducibility and the lower time spent for each analysis. These improvements have made possible the evaluation and/or quantification of the complex anaerobic microbial communities involved in AD as well as the observation of their change when the AD conditions are modified, e.g. the addition of a co-substrate.

At present, it is still not clear how the microbial community structure influences digester performance. Nonetheless, it is accepted that it is greatly influenced by the digester feedstock and operational conditions. As a general trend, the publications analyzing the microbial dynamics when AcoD was applied, suggested that the substrate diversification and the better nutritional balance led to a more versatile and robust microbial community. Therefore, they were able to withstand a wider range of operational conditions or perturbations and even enhance biogas production [153–156]. The AD process has been typically divided into four metabolic steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis; however, from a microbiological point of view, AD can also be divided according the functionality of its major microbial domains, *Bacteria* and *Archaea*. On the one hand, bacterial consortia take part into the hydrolysis, the acidogenesis and the acetogenesis step; therefore, *Bacteria* have the capacity to metabolize a large variety of substrates and in a wide range of operational conditions. It has been stated that the most important phyla involved in the AcoD processes are *Firmicutes*, especially the *Clostridiales* order, *Bacteroidetes*, and *Actinobacteria*. On the other hand, the archaeal consortia, especially the methanogens, hold a key role in the AD process, since they are responsible for methane formation.

Temperature is one of the main factors influencing the bacterial consortia in AD. Ziganshin et al. [157] showed the bacterial composition shift when the temperature of a digester treating CM and dried

distiller grains was changed from mesophilic to thermophilic conditions. To be specific, the temperature change from 38 to 55 °C led to a decrease in several *Bacteroidetes* phylotypes and an increase in the abundance of unclassified *Clostridiales*. Ziganshin et al. [157] also reported that, when treating agro-industrial waste, the most abundant bacterial phyla were *Firmicutes* (mainly *Clostridia*) and *Bacteroidetes*. These results are in agreement with Martin-Gonzalez et al. [158], who reported *Firmicutes* as the major phylum in a digester treating OFMSW and FOG at thermophilic conditions. Another important factor affecting the bacterial diversity is the feedstock composition. Wang et al. [155] observed how the microbial community remained stable when a digester was mono-digesting CM and co-digesting up to 20% (VS basis) of grass silage in feedstock. Nevertheless, they also recorded a diversification of the bacterial community when the grass silage supplied the 30 and 40% of VS present in the influent. Likewise, Ziganshin et al. [157] found different bacterial consortia depending on the substrate features. For instance, when treating *Jatropha* press cake, a fiber-rich waste, an enriched fiber-specialized consortia belonging to phyla *Actinobacteria* and *Fibrobacteres* were dominant. Other important parameters that can influence the bacterial composition are the ORL or the HRT. Under non-stress conditions, Yue et al. [159], who co-digested CM with corn stover, observed that at high HRT (50 days) the order *Clostridiales* was the main bacteria, while at lower HRT (40 and 30 days) the phylum *Bacteroidetes* co-dominate the bacterial consortia along with the *Clostridiales*. The comparison between a single-phase and a two-phase AcoD system treating PM and cassava-pulp, showed that *Firmicutes* were the exclusive dominant phylum in the hydrolytic/acidogenic reactor (2 days HRT and pH of 4.5), whereas in the single-phase AcoD (15 days) *Bacteroidetes* were found in great number [160]. Finally, overloaded conditions led to a diminution of the bacterial diversity as well as a shift in phylum dominance [18,153,155].

Methanogenic *Archaea* can be classified depending on the two main substrates used for methane production: (i) the strictly aceticlastic methanogens, which comprise the genus *Methanosaeta*, and (ii) the hydrogenotrophic methanogens comprising the orders of *Methanobacteriales*, *Methanomicrobiales* and *Methanococcales*. Moreover, the genus *Methanosarcina* is considered a mixotrophic methanogen since can use either acetate or H₂/CO₂ to produce methane. It is well known that methanogens are the most susceptible microbes in the AD process to environmental and operational conditions changes. As for *Bacteria*, temperature is one of the most important factors that can cause a shift in methanogenic consortia [161,162]. Goberna et al. [161], who co-digested CM and OMW, found a decrease of *Methanosarcina* when changing the temperature from 37 to 55 °C, favoring a rising in less-frequent populations such as *Methanoculleus* (*Methanomicrobiales* order), *Methanobacterium* and *Methanothermobacter* (*Methanobacteriales* order), and an uncultured methanogens. Actually, similar methanogens were detected by Martin-Gonzalez et al. [158] when treating OFMSW and FOG at thermophilic conditions. Treating a mixture of CM and dried distiller grains, Ziganshin et al. [157] observed how the *Methanosaeta*, predominant at mesophilic conditions, disappeared and instead *Methanosarcina* emerged at thermophilic condition. The temperature shift also led to a considerable decrease of *Methanoculleus* and *Methanomethylovorans*. In fact, Ziganshin et al. [157] linked the increase of the *Methanosarcina* numbers with the higher VFA concentration recorded at thermophilic conditions. This result is in accordance with previous findings which stated that *Methanosaeta* and *Methanosarcina* dominate as a function of the VFA and ammonia concentration in the digester medium [163]. Karakashev et al. [163] observed that *Methanosaeta* dominate in media with low VFA and ammonia levels whereas *Methanosarcina* dominate when the VFA or the ammonia concentration are moderate, probably due to their morphology.

It is clear that changes in the feedstock composition can modify the digester medium, such as VFA and ammonia concentration, and therefore drive to changes in the methanogenic composition.

For example, the addition of a co-substrate that increased the ammonia levels leading to a shift in methanogens, usually dominating the *Methanosarcina* [164,165]. Moreover, it has been found that high ammonia levels and/or high VFA concentrations can cause the acetoclastic methanogen inhibition, and a further change in the acetate metabolic pathway. In some cases, acetate can be oxidized into H_2 and CO_2 by syntrophic acetate bacteria; hence, a hydrogenotrophic methanogen partner is needed to complete the methanogenesis [166–168]. Ziganshin et al. [157] supported this premise with their results when co-digesting CM and chicken manure at mesophilic conditions and an ammonia level of 5 kg m^{-3} . They reported the presence of some *Clostridiales* able to perform syntrophic acetate oxidizing activity and the dominance of *Methanoculleus*, which are hydrogenotrophic methanogens, while acetoclastic methanogens were not detected. In this vein, the most common methanogen found under ammonia or VFA stress is *Methanoculleus*, suggesting that they can tolerate higher concentrations of intermediate digestion products [153,157,161,164,165]. Finally, although its role in the AD process remains unknown, *Crenarcheota* (another *Archaea* phylum) has been detected in some AcoD assay [156,157,165].

6.1. Biomass dynamics in full-scale plants

Microbial community behavior at full-scale plants showed similar trend than those obtained from lab-scale experimentation. In a Thailand plant (2400 m^3) operated at mesophilic conditions, Supaphol et al. [154] evaluated how the three different stages in which the AD process is separated influenced bacterial and archaeal community. For example, the addition of night soil waste into a mixture of MSW and FVW enhanced the numbers of *Anaerovorax* (*Clostridiales* order), diversifying the microbial consortia. Nevertheless, *Methanosaeta* remained as the dominant methanogen during the whole process. Regarding archaeal community, St-Pierre and Wright [169], who evaluated the anaerobic manure digesters operated on three dairy farms in United States, found higher methanogenic diversity when CM was co-digested with oil fish waste rather than with dairy wastes. It was suggested that the diversification could be linked to high lipid content of the oily residue [169]. Likewise, Sundberg et al. [162], who studied the microbial community of 21 full-scale plants (14 of them devoted to AcoD), coupled the digesters in three groups depending on the communities at genus level: (i) SS mono-digesters, (ii) mesophilic AcoD digesters, and (iii) thermophilic AcoD digesters. Consequently, the authors concluded that the microbial patterns were mainly affected by substrate composition and process temperature. These results are in agreement with those reported by Regueiro et al. [170] who, after analyzing six mesophilic full-scale digesters, found that the microbial community was dependent on the substrate nature. Sundberg et al. [162] results showed that the AcoD digesters had lower microbial diversity than SS digesters. That fact was related with the hygienization pre-treatment ($70\text{ }^\circ\text{C}$, 1 h) carried out in most AcoD plants and the enrichment done by mixing PS and WAS in the SS digesters.

7. Anaerobic co-digestion modeling

In later years (2010–2013), not much attention has been paid to AcoD modeling. It is indeed very surprising that only few articles among the more than two hundreds AcoD articles published within this period were devoted to this topic. It should be pointed out that reliable AcoD modeling is required to predict, in a clear and quantifiable manner, the effect of mixing two or more wastes in a digester and remove potentially negative impacts from mixing based on random or heuristic decisions [3,25]. Moreover, modeling development may reduce the time and money associated with

laboratory experiments as well as to improve co-substrate selection and dosage rates [171,172].

The IWA Anaerobic Digestion Model no. 1 (ADM1) [173], designed to be easy to extend, provides a common biochemical conversion structure, mass balance equations, process kinetics and stoichiometry for AD modeling. Since 2002, ADM1 has become the common framework in AcoD models, MATLAB/SIMULINK[®] the most used software being to solve the differential algebraic equations of the modified ADM1 models [25,174–177]. In this matter, the two premises cited in our previous review for AcoD models are still valid [3]: (i) substrate characterization should be in terms of carbohydrates, proteins, lipids and inerts, and (ii) the disintegration/hydrolysis step is generally considered the rate-limiting step.

Regarding the mixture between SS and OFMSW, Esposito et al. [174,175] further developed the model described by the same authors [171]. In this model, SS is disintegrated using a first-order kinetics while OFMSW disintegration is simulated using a surface-based kinetics as for Sanders et al. [178]. According to the authors, the advantage of the surface-based kinetics over the conventional first-order kinetics is that the surface-based constant only depends on the nature of the OFMSW, whereas the first-order kinetics depends on both nature and particle size distribution of the OFMSW. However, the surface-based kinetics requires the addition of a second parameter (a^* , i.e. the overall surface area of the particles to be disintegrated per unit mass), which depends on the particle size distribution of the OFMSW [174]. Moreover, their last publication included [175] (i) the LCFA into the charge balance to better simulate pH inhibition and (ii) the possibility of splitting disintegration products (carbohydrates, proteins and lipids) in two fractions, i.e., a readily biodegradable fraction and a slowly biodegradable fraction. Garcia-Gen et al. [179] who co-digested a mixture of FVW have also implemented this latter approach, which supposes the addition of three extra processes in the Petersen matrix and the pertinent first-order hydrolysis constants.

Astals et al. [25] and Zhou et al. [176] applied, with different purposes, the ADM1 to model the AcoD of agro-industrial wastes. Astals et al. [25], who used the modified ADM1 model for agro-wastes developed by Gali et al. [172], evaluated the feasibility of mixing pig manure and glycerol, while focusing on nitrogen limitation. Gali et al. [172] model uploads, although glycerol is a soluble substrate, both substrates as particulate organic matter. Adding glycerol as carbohydrates avoided the further extension of the Petersen matrix of a model devoted to solid wastes. However, the model did not adapt to reality since glycerol is degraded through Monod kinetics instead of first-order kinetics. Another option to incorporate soluble substrates not described in the ADM1 is the one suggested by Garcia-Gen et al. [177], who developed a methodology to convert soluble fermentable compounds into equivalents of glucose. Thus, each substrate fermentation catabolic yields are function of the standard ADM1 sugar fermentation yields [177]. Finally, Zhou et al. [176] assessed the feasibility of three AcoD mixtures (i.e. manure and OFMSW, manure and corn silage and OFMSW and corn silage) under different operating (HRT and OLR) conditions. The authors also developed an input–output feedback control based on methane production to maintain the manure and corn silage co-digester stable based on the results of simulation. To be specific, the methane and biogas flow together with the VFA concentration were the variables used by the control system to produce an output and keep the desired operation conditions.

8. Conclusions

Anaerobic co-digestion has been widely implemented in the rural sector because of the need to improve digesters biogas production. Agro-industrial waste and the organic fraction of the municipal solid waste are the most reported co-substrate for

manure-based digesters. However, easily degradable substrates are currently limited. Therefore, more complex waste have been considered, which raised the interest on pre-treatments. Even though pre-treatments need to be further developed, because of the upcoming possibilities, steam explosion and low temperature pre-treatments have shown the best results. Nonetheless, pre-treatments selection and dose, and therefore its feasibility, depend on several factors and not only on the increase of the methane yield. Future implementation of AcoD in the agriculture area is linked to the development of economically self-sustainable systems; in this regard over the last years many studies have been devoted to: (i) maximizing the biogas production by applying optimal conditions, (ii) utilizing new residues via pre-treatments, and (3) increasing the revenues in the plant by the digestate application as fertilizer. The main risk over digestate quality when co-digestion is implemented is the production of unstable digestates, which is the result of the prevalence of the efficiency criteria for biogas production over digestate stability.

Sewage sludge stands the second as a main substrate for anaerobic co-digestion. Although the mixture between sewage sludge and the organic fraction of the municipal solid waste had been the most reported co-digestion mixture, between 2010 and 2013 the publication dealing with in-house waste has risen. Anaerobic co-digestion between sludge and fat, oil and grease from the grit chamber is an interesting approach because of the lipids high methane potential. However, more clarifying data is required to identify the optimal operational conditions and to reduce long chain fatty acids inhibition. Algae, although having a lower methane potential, have also been widely applied as a co-substrate. Their potential lay in the fact that their growing can be used as a post-treatment for wastewaters nutrient removal, which may also reduce the impact of the returning side-streams to the head of the wastewater treatment plant.

The organic fraction of the municipal solid waste as a main substrate has not been as studied as manures or sewage sludge. Actually, it has widely been used as co-substrate for manure and sewage sludge digesters, probably due to the presence of undesired compounds. Therefore, there is a lack of reported information with reference to the co-digestion of biowaste with other waste.

Concerning microbial dynamics, it has been observed that methanogens dynamics are more affected by volatile fatty acids and ammonia concentration than by the addition of a co-substrate. In contrast, although the lack of knowledge about some uncultured bacteria is a handicap when dealing with complex anaerobic consortia, bacteria population seems to be more affected than methanogens by the addition of a co-substrate. As a general trend, co-digestion (substrate diversification) led to a more versatile and robust microbial community. However, more knowledge is needed about how microbial population is affected by the addition of a co-substrate, in terms of kinetics, stability and yields.

Considering that modeling development may reduce the time and money associated with laboratory experiments as well as to improve co-substrate selection and dosage rates, more efforts should be made to improve ADM1 to better predict the interactions that take place under co-digestion. There is a broad need for more published data from “oriented research”, which could lead to the improvement of modeling for a more accurate prediction of the impact of co-digestion.

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